Marine Natural Products. XXVIII.[1] The Structures of Sarasinosides A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, and C_3, Nine New Norlanostane-Triterpenoidal Oligoglycosides from the Palauan Marine Sponge *Asteropus sarasinosum*

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The chemical structures of sarasinosides A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, and C_3, nine 30-norlanostane-triterpenoidal oligoglycosides isolated from the Palauan marine sponge *Asteropus sarasinosum* have been elucidated on the basis of chemical and physicochemical evidence. Sarasinosides A_2 (2) and A_3 (3) were shown to be the 7,9(11)-diene and 8,14-diene analogs of sarasinoside A_1 (1), whereas sarasinosides B_2 (7) and B_3 (9) were the 7,9(11)-diene and 8,14-diene analogs of sarasinoside B_1 (5), respectively. Similar structural correlations of sarasinosides C_2 (6) and C_3 (8) with sarasinoside C_1 (4) were demonstrated. These sarasinosides characteristically contain one mole each of *N*-acetylglucosamine and *N*-acetylgalactosamine in their oligosaccharide moieties.

Keywords sarasinoside A_1; sarasinoside A_2; sarasinoside A_3; sarasinoside B_1; sarasinoside C_1; marine saponin; norlanostane triterpene oligoglycoside; marine sponge; *Asteropus sarasinosum*; *N*-acetylglucosamine

Saponins occur very widely in the plant kingdom,[2,3] and among marine organisms, sea cucumber and starfish characteristically produce these saponins. Namely, sea cucumber produces lanostane-triterpenoidal oligoglycosides, while starfish metabolites steroidal oligoglycosides.

During the course of our studies in search of new biologically active marine natural products,[3] we have investigated the chemical constituents of the Palauan marine sponge *Asteropus sarasinosum* and isolated nine new ichthyotoxic norlanostane-triterpenoidal oligoglycosides named sarasinosides A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**5**), B_2 (**7**), B_3 (**9**), C_1 (**4**), C_2 (**6**), and C_3 (**8**).[4] This was the first isolation of saponins from a marine organism other than sea cucumber and starfish (echinoderm). These sarasinosides character-

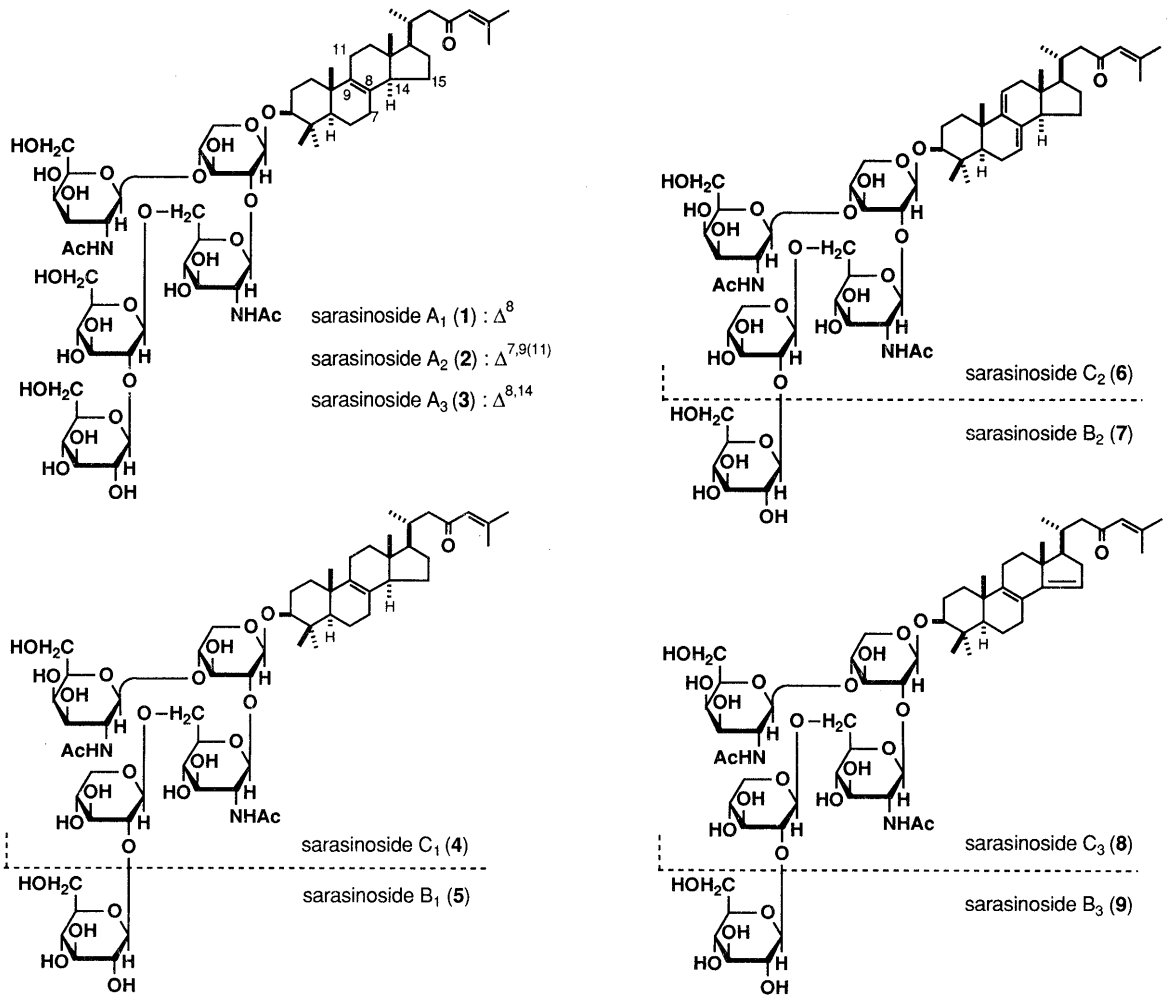


Chart 1

istically contain one mole each of *N*-acetylglucosamine and *N*-acetylgalactosamine in their oligosaccharide moieties. In a previous paper,[4] we reported the isolation of these nine oligoglycosides and the chemical structures of three major oligoglycosides: sarasinosides A_1 (**1**), B_1 (**5**), and C_1 (**4**).[5] This paper presents a full account of the chemical evidence supporting the proposed structures of sarasinosides A_1 (**1**), B_1 (**5**), and C_1 (**4**) and also deals with the structure elucidation of six congeneric oligoglycosides: sarasinosides A_2 (**2**), A_3 (**3**), B_2 (**7**), B_3 (**9**), C_2 (**6**), and C_3 (**8**).

The freeze-dried sponge, which was collected at -5 m in the Palau Islands, was extracted with methanol (MeOH) and the extract was partitioned into a mixture of ethyl acetate and water. The water-soluble portion was then partitioned into a mixture of 1-butanol and water. Chromatographic separation of the 1-butanol soluble portion provided three saponin fractions containing sarasinosides A, B, and C, which each gave a single spot on a thin-layer chromatogram (TLC). Each fraction was further separated by high-performance liquid chromatography (HPLC) finally to afford sarasinosides A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**5**), B_2 (**7**), B_3 (**9**), C_1 (**4**), C_2 (**6**), and C_3 (**8**) in 0.68, 0.25, 0.40, 0.50, 0.17, 0.33, 0.13, 0.04, and 0.10% yields, respectively, from the 1-butanol soluble portion.

Sarasinosides A_1 (1), B_1 (5), and C_1 (4) Sarasinoside A_1 (**1**), the major oligoglycoside, was obtained as fine crystals of mp 207—210 °C. In the infrared (IR) spectrum, it showed strong absorption bands at 3370 (br) and 1067 (br) cm^{-1}, indicative of glycosidic structure. Sarasinoside A_1 (**1**) showed an ultraviolet (UV) absorption maximum at 237 nm ($\varepsilon = 13500$) which was ascribable to a conjugated enone chromophore. The presence of this conjugated enone moiety was also suggested by the carbon-13 signals observed at δ_C 200.5 (s, C-23), 124.8 (d, C-24), and 153.7 (s, C-25), together with the signals of two olefinic carbons observed at δ_C 127.5 (s, C-8) and 136.4 (s, C-9) in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum.

Acidic hydrolysis of sarasinoside A_1 (**1**) with 20% aqueous H_2SO_4–MeOH (1 : 1) yielded two secondarily formed sapogenols **10** and **11**. The proton nuclear magnetic resonance (^{1}H-NMR) decoupling experiments and the ^{13}C-NMR analysis of **10** and **11** led us to presume that **10** and **11** were double bond isomers having a norlanostane skeleton.[6] The relative configuration of **11** having a Δ^{14} double bond was established by X-ray crystallographic analysis.[4] The ^{1}H- and ^{13}C-NMR spectra of **11** showed the signals due to the Δ^{14} double bond at δ 5.13 (m, 15-H) and δ_C 155.8 (s, C-14), 117.2 (d, C-15) together with the signals assignable to the conjugated enone moiety in the side chain at δ 6.07 (s, 24-H) and δ_C 200.6 (s, C-23), 125.0 (d, C-24), and 153.9 (s, C-25). The ^{13}C-NMR spectrum of the other sapogenol **10** showed the signals of two olefinic carbons, which were assigned to the $\Delta^{8(14)}$ double bond, at δ_C 127.1 (s, C-8) and 141.8 (s, C-14), and the signals of the conjugated enone moiety in the side chain at δ_C 200.6 (s, C-23), 125.0 (d, C-24), and 153.9 (s, C-25). On the other hand, the ^{13}C-NMR signals assignable to the tetrasubstituted olefinic carbons in the aglycone part of sarasinoside A_1 (**1**) were observed at δ_C 127.5 (s) and 136.4 (s) as mentioned above. Interestingly, acidic hydrolysis of **1** under milder conditions, *e.g.* 10% H_2SO_4–MeOH (1 : 1) provided the sapogenol **10** as a single product. Comparison in detail

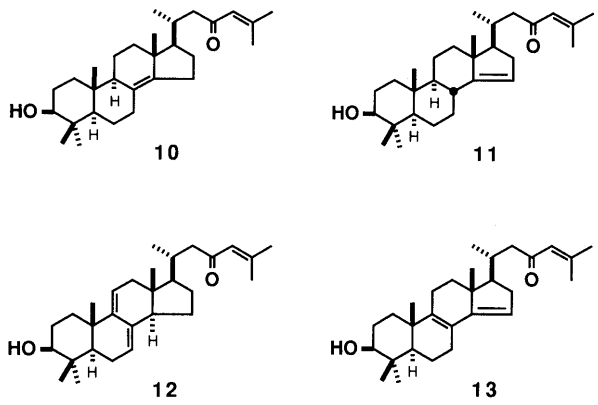


Chart 2

of the ^{13}C-NMR data for **1**, **10**, and **11** with those for known compounds[6] led us to conclude that the genuine aglycone of **1** has a Δ^8 double bond as depicted in **14**, and the acidic hydrolysis of **1** gave rise to double bond migration in the aglycone part to liberate the artifactual sapogenols **10** and **11** having a thermodynamically more favored double bond.[7]

Sarasinoside A_1 (**1**) is a pentaglycoside comprising 2 mol of D-glucose and 1 mol each of D-xylose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine in its oligosaccharide portion. The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals at δ 5.58 (d, $J = 8.6$ Hz), 5.56 (d, $J = 8.5$ Hz), 5.20 (d, $J = 7.6$ Hz), 5.15 (d, $J = 8.6$ Hz), and 4.53 (d, $J = 8.2$ Hz) and at δ_C 106.7, 105.5, 102.6, 102.2, and 101.7 (each d) which suggested the β-anomeric configurations of these five monosaccharide constituents. In order to determine the sequence in the oligosaccharide portion, **1** was subjected to enzymatic hydrolysis with crude hesperidinase to afford two prosapogenols, A_1-pro-1 (**15**) and A_1-pro-2 (**16**).

A_1-pro-1 (**15**) was a diglycoside having 1 mol each of xylose and *N*-acetylglucosamine. Methanolysis of fully methylated A_1-pro-1, which was prepared by complete methylation of **15**, liberated methyl 3,4-di-*O*-methylxylopyranoside. Thus, the structure of A_1-pro-1 has been determined as **15**.

A_1-pro-2 (**16**) was a tetraglycoside having 1 mol each of xylose, glucose, *N*-acetylglucosamine, and *N*-acetylgalactosamine in its carbohydrate portion. Methanolysis of fully methylated A_1-pro-2, which was also obtained by complete methylation of **16**, liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3-*O*-methylxylopyranoside. Another enzymatic hydrolysis of **1** using β-glucosidase (type II from almonds) gave another tetraglycoside A_1-pro-3 (**17**) which comprised 2 mol of glucose and 1 mol each of xylose and *N*-acetylglucosamine in its carbohydrate portion. Complete methylation of **17** followed by methanolysis liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside, and methyl 3,4-di-*O*-methylxylopyranoside. In the ^{13}C-NMR spectrum of **16** and **17**, the signals of C-6″ in the *N*-acetylglucosamine moieties were observed at a lower field (δ_C 69.8 in **16** and δ_C 69.6 in **17**) than the C-6″ signal of **15** (δ_C 62.5) due to the glycosidation shift.[8,9a] Based on the foregoing evidence, the structures of A_1-pro-2 and A_1-pro-3 have been determined as **16** and **17**, respectively.

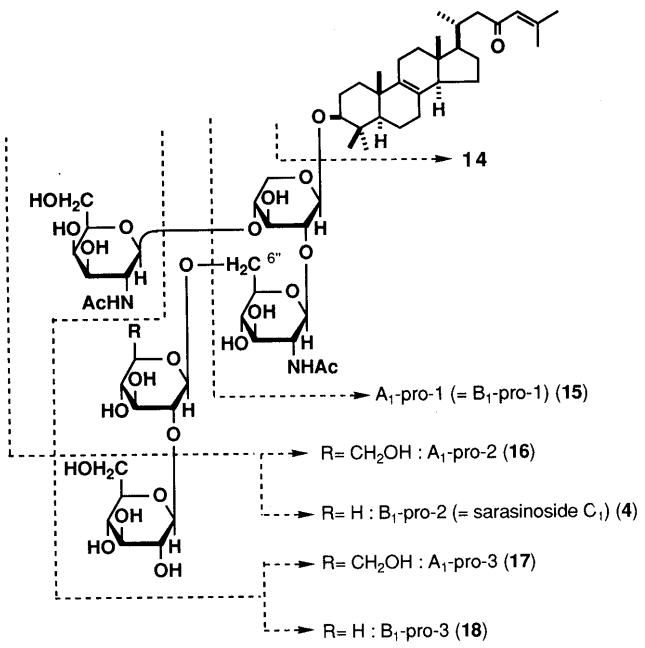


Chart 3

In a similar manner, complete methylation followed by methanolysis of sarasinoside A_1 (**1**) liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside, and methyl 3-*O*-methylxylopyranoside. Consequently, the structure of sarasinoside A_1 (**1**) has been determined to be as shown. In the ordinary methylation analysis of **1** and its prosapogenols (**15**, **16**, **17**) mentioned above, unambiguous results in regard to the branching at the *N*-acetylamino sugar moieties were not obtained. A similar result was also experienced in the following structure elucidation of sarasinoside B_1 (**5**). Direct chemical evidence on the branching at the *N*-acetylamino sugar moiety has been obtained by other means as described later.

Sarasinoside B_1 (**5**) was obtained as fine crystals of mp 197—199 °C. It showed a UV absorption maximum at 239 nm ($\varepsilon = 13600$) similarly to sarasinoside A_1 (**1**). Sarasinoside B_1 (**5**) is a pentaglycoside containing 2 mol of D-xylose and 1 mol each of D-glucose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine. Acidic hydrolysis of **5** yielded two artifactual sapogenols **10** and **11** as in the case of sarasinoside A_1 (**1**). The ^{13}C-NMR spectrum of **5** showed the Δ^8 olefinic carbon signals at δ_C 127.7 (s, C-8) and 136.4 (s, C-9) together with many other signals closely resembling those of **1**, except for some signals due to the oligosaccharide moiety. Thus, the genuine aglycone of sarasinoside B_1 (**5**) has been shown to be identical with that of sarasinoside A_1 (**1**).

The ^{1}H- and ^{13}C-NMR spectra of sarasinoside B_1 (**5**) showed signals at δ 5.56 (d, $J = 8.5$ Hz), 5.43 (d, $J = 7.3$ Hz), 5.21 (d, $J = 7.3$ Hz), 5.20 (d, $J = 8.2$ Hz), and 4.57 (d, $J = 7.6$ Hz) and at δ_C 106.7, 105.5, 103.2, 102.3, and 102.0 (each d), which suggested the β-anomeric configurations of the five monosaccharide moieties in **5**. Enzymatic hydrolysis of **5** using crude hesperidinase furnished B_1-pro-1 ($= A_1$-pro-1) (**15**) and B_1-pro-2 (**4**), whereas another enzymatic hydrolysis of **5** using β-glucosidase afforded B_1-pro-3 (**18**). Among these partial hydrolysates, B_1-pro-1 was shown to be identical with A_1-pro-1 (**15**).

Acidic hydrolysis of the prosapogenol B_1-pro-2 (**4**), a tetraglycoside, furnished 2 mol of xylose and 1 mol each of *N*-acetylglucosamine and *N*-acetylgalactosamine. Methanolysis of fully methylated B_1-pro-2, prepared by complete methylation of **4**, liberated methyl 2,3,4-tri-*O*-methylxylopyranoside and methyl 3-*O*-methylxylopyranoside. On the other hand, acidic hydrolysis of B_1-pro-3 (**18**) furnished 2 mol of xylose and 1 mol each of glucose and *N*-acetylglucosamine. Complete methylation of **18** followed by methanolysis liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methylxylopyranoside. Based on the above-mentioned evidence, the structures of B_1-pro-2 and B_1-pro-3 have been determined as **4** and **18**, respectively.

Furthermore, complete methylation of sarasinoside B_1 (**5**) followed by methanolysis liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4-di-*O*-methylxylopyranoside, and methyl 3-*O*-methylxylopyranoside. Consequently, the structure of sarasinoside B_1 (**5**) has been concluded to be as shown.

Sarasinoside C_1 was a tetraglycoside containing 2 mol of xylose and 1 mol each of *N*-acetylglucosamine and *N*-acetylgalactosamine in its carbohydrate portion. Acidic hydrolysis of sarasinoside C_1 (**4**) provided two artifactual sapogenols **10** and **11**, as were obtained above on the acidic hydrolysis of sarasinosides A_1 (**1**) and B_1 (**5**). Eventually, sarasinoside C_1 was shown to be identical with B_1-pro-2 (**4**).

As mentioned above in connection with the methylation analysis of sarasinosides A_1 (**1**), B_1 (**5**), and C_1 (**4**), rather complicated products were obtained from the *N*-acetylamino sugar moieties. Thus, in the initial complete methylation, *N*-methylation of *N*-acetylglucosamine and *N*-acetylgalactosamine moieties occurred, so that subsequent methanolysis of the fully methylated derivatives of **1**, **5**, and **4** gave rise to ready cleavage of the *N*-acetamide linkage to yield HCl salts of *N*-methylglucosamine and *N*-methylgalactosamine derivatives. These co-occurring reactions presumably resulted in the failure of the gas-liquid chromatographic (GLC) and TLC analyses of the methylated *N*-acetylglucosamine and *N*-acetylgalactosamine moieties.

In order to get more direct proof of the carbohydrate sequence of the *N*-acetylamino sugar moieties, fully methylated derivatives of sarasinosides A_1 (**1**) and B_1 (**5**) were subjected to Hakomori's degradation method.[10] Thus, the fully methylated derivatives of **1** and **5** were each heated with H_2SO_4–AcOH. Then, the reaction mixture was diluted with water and the whole mixture was heated again. The hydrolysates thus obtained were treated with sodium borohydride and subsequently acetylated with acetic anhydride. After purification, partially methylated hexitol acetates were obtained as follows: 3-*O*-methyl-1,2,4,5-tetra-*O*-acetylxylitol (**19**), 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylglucitol (**20**), 3,4,6-tri-*O*-methyl-1,2,5-tri-*O*-acetylglucitol (**21**) from **1**; **19**, **20**, 3,4-di-*O*-methyl-1,2,5-tri-*O*-acetylxylitol (**22**) from **5**; and 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidogalactitol (**23**) and 3,4-di-*O*-methyl-1,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol (**24**) from both **1** and **5**. The latter *N*-methylacetamidohexitols, **23** and **24**, were identified by direct comparisons with authentic samples which were synthesized from *N*-acetyl-D-glucosamine and *N*-acetyl-D-galac

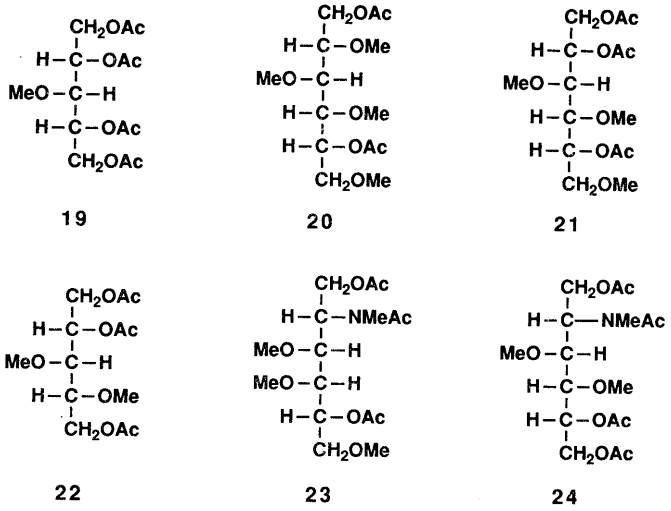
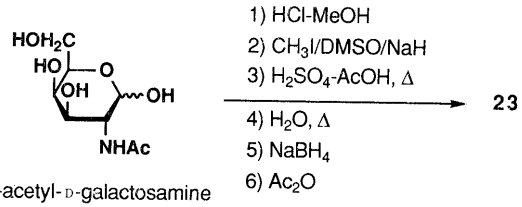


Chart 4



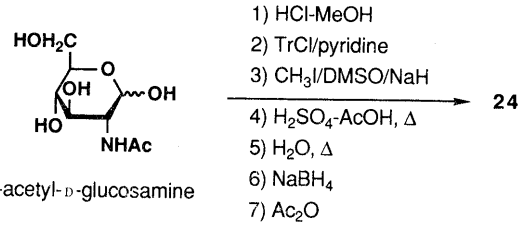


Chart 5

tosamine through the procedures shown in Chart 5. The ^{1}H-NMR spectra of **23** and **24** showed that **23** and **24** were each present as a mixture of two conformers in solution.

Sarasinosides A_2 (2) and A_3 (3) Sarasinoside A_2 (**2**) and sarasinoside A_3 (**3**), which moved with the same *Rf* value as sarasinoside A_1 (**1**) on ordinary TLC, were obtained as fine crystals having the same melting point, 205—208 °C. Sarasinoside A_2 (**2**) showed UV absorption maxima at 239 (shoulder) nm (ε=22000), 243 (23500), 251 (sh) (16500) and an IR absorption band at 1630 (br) cm^{-1}, which were attributable to a conjugated enone and *N*-acetylamino moieties. The IR spectrum of **2** also showed strong, broad absorption bands at 3360 and 1050 cm^{-1} due to the glycosidic structure (Table I). The circular dichroism (CD) spectrum of **2** showed complex maxima ($[\theta]_{234}$ +6200, $[\theta]_{248}$ −3500, $[\theta]_{256}$ −3200) ascribable to the conjugated diene and conjugated enone moieties in **2**.

Acidic hydrolysis of sarasinoside A_2 (**2**) with 3% aqueous H_2SO_4 provided another sapogenol **12**. The sapogenol (**12**) showed UV absorption maxima at 239 (sh) nm (ε=22900),

Table I. UV, CD, and ^{13}C-NMR Data for Sarasinosides A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**5**), B_2 (**7**), B_3 (**9**), C_1 (**4**), C_2 (**6**), C_3 (**8**) and Sapogenols (**12**, **13**)

Compound	UV λ_{max} nm (ε)	CD $[\theta]_{nm}$ (peak or trough)	^{13}C-NMR δ_C (Olef. C of sapogenol)
Sarasinoside A_1 (**1**)	237 (13500)	222 (−6600)	127.5 (s), 136.4 (s)
Sarasinoside A_2 (**2**)	239sh(22000) 243 (23500) 251sh(16500)	234 (+6200) 248 (−3500) 256 (−3200)	117.2 (d), 122.0 (d) 135.8 (s), 147.3 (s)
Sarasinoside A_3 (**3**)	245 (22000)	231 (−15900)	116.9 (d), 122.8 (s) 142.3 (s), 151.4 (s)
Sarasinoside B_1 (**5**)	239 (13600)	222 (−7200)	127.7 (s), 136.4 (s)
Sarasinoside B_2 (**7**)	239sh(21700) 243 (22800) 251sh(16200)	233 (+8000) 248 (−3500) 256 (−4000)	117.2 (d), 121.9 (d) 135.8 (s), 147.2 (s)
Sarasinoside B_3 (**9**)	243 (21600)	231 (−14800)	116.9 (d), 122.8 (s) 142.1 (s), 151.2 (s)
Sarasinoside C_1 (**4**)	237 (13600)	222 (−6500)	127.3 (s), 135.9 (s)
Sarasinoside C_2 (**6**)	239sh(22500) 243 (23800) 251sh(16800)	234 (+4700) 248 (−5000) 255 (−4400)	117.0 (d), 121.7 (d) 135.8 (s), 147.0 (s)
Sarasinoside C_3 (**8**)	244 (25900)	231 (−15900)	116.7 (d), 122.7 (s) 141.9 (s), 151.1 (s)
12	239sh(22900) 243 (24000) 250sh(17800)	229 (+1200) 247 (−2700) 254 (−2200)	117.4 (d), 121.9 (d) 136.1 (s), 147.3 (s)
13	243 (33300)	231 (−5500)	117.1 (d), 123.1 (s) 142.5 (s), 151.6 (s)

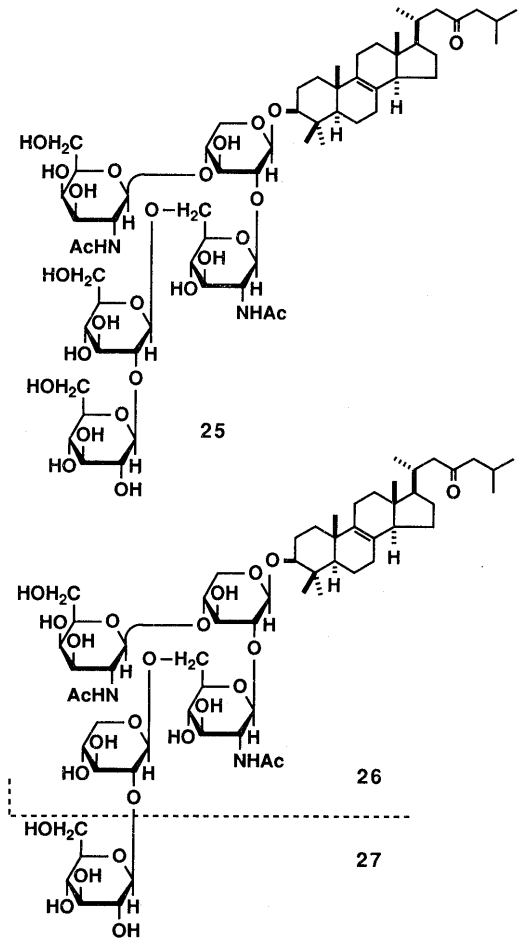


Chart 6

243 (24000), 250 (sh) (17800) and complex CD maxima ($[\theta]_{229}$ +1200, $[\theta]_{247}$ −2700, $[\theta]_{254}$ −2200) which were similar to those observed for **2**. The ^{1}H-NMR spectrum of **12** showed four singlet methyl signals at δ 0.55, 0.90, 0.98, and 1.01, a doublet methyl signal at δ 0.95 (J=6.4 Hz), and two olefinic proton signals at δ 5.40 and 5.45 (both m) together with the signals of an olefinic proton [δ 6.06 (s)] and two olefinic methyls [δ 1.89, 2.15 (both, s)] ascribable to the 24-en-23-one structure in the side chain. The ^{1}H- and ^{13}C-NMR analysis[6] in detail of **12** disclosed that **12** was a 7,9(11)-diene analog of the above-described norlanostane-type sapogenol **11**, which was obtained by the acidic hydrolysis of sarasinoside A_1 (**1**) and the structure of which had been determined by X-ray crystallographic analysis.[4]

The carbon signals assignable to the sugar moiety in the ^{13}C-NMR spectrum of sarasinoside A_2 (**2**) were shown to be superimposable on those of sarasinoside A_1 (**1**). So, **2** was presumed to be a 7,9(11)-diene analog of **1**. In order to verify this presumption, sarasinoside A_2 (**2**) was subjected to catalytic hydrogenation over 10% palladium–carbon to afford a tetrahydro derivative **25**, which was shown to be identical with a reduction product prepared from sarasinoside A_1 (**1**) under the same reaction conditions. Based on the combined evidence, the structure of sarasinoside A_2 (**2**) has been determined to be as shown.

Sarasinoside A_3 (**3**) showed a UV absorption maximum at 245 nm (ε=22000) and a negative CD maximum at $[\theta]_{231}$ −15900 due to its conjugated diene and conjugated enone chromophores. Acidic hydrolysis of sarasinoside A_3 (**3**) with 5% aqueous H_2SO_4–MeOH (1 : 1) furnished another sapogenol **13**. The sapogenol (**13**) showed a UV absorption maximum at 243 nm (ε=33300) and a negative CD maximum ($[\theta]_{231}$ −5500) similar to those of the parent glycoside sarasinoside A_3 (**3**). The ^{1}H-NMR spectrum of **13** showed four singlet methyl signals at δ 0.84, 0.86, 1.02, and 1.04, a doublet methyl signal at δ 0.97 (J=6.4 Hz) and one olefinic proton signal at δ 5.34 (m) together with the signals of an olefinic proton [δ 6.08 (s)] and two olefinic methyls [δ 1.89, 2.15 (both s)], which were ascribable to the 24-en-23-one structure as seen in the aglycone parts of sarasinoside A_1 (**1**) and A_2 (**2**). The ^{1}H-NMR decoupling experiments and the ^{13}C-NMR analysis in detail of **13** led us to presume that **13** was an 8,14-diene isomer of the sapogenol **12**. The carbon signals assignable to the aglycone part in the ^{13}C-NMR spectrum of sarasinoside A_3 (**3**) were very similar to those of **13**, whereas the carbon signals assignable to the sugar moiety were shown to be superimposable on those of sarasinoside A_1 (**1**). These findings indicated that **3** was an 8,14-diene analog of **1**. In order to verify this, sarasinoside A_3 (**3**) was subjected to catalytic hydrogenation over 10% palladium–carbon to afford the tetrahydro derivative **25**, which was identical with the reduction product obtained above from sarasinoside A_1 (**1**). Consequently, the chemical structure of sarasinoside A_3 has been determined to be **3**.

Sarasinosides B_2 (7) and C_2 (6) Sarasinoside B_2 (**7**) and sarasinoside C_2 (**6**) were obtained as fine crystals of mp 192—195 °C and mp 188—191 °C, respectively. The ^{13}C-NMR spectra of **7** and **6** closely resembled the spectrum of sarasinoside A_2 (**2**) except for some signals due to the oligosaccharide moieties. Sarasinosides B_2 (**7**) and C_2 (**6**) showed characteristic UV absorption maxima: 239 (sh) nm (ε=21700), 243 (22800), 251 (sh) (16200) for **7**; 239 (sh) (22500), 243 (23800), 251 (sh) (16800) for **6**, and CD maxima: $[\theta]_{233}$ +8000, $[\theta]_{248}$ −3500, $[\theta]_{256}$ −4000 for **7**; $[\theta]_{234}$ +4700, $[\theta]_{248}$ −5000, $[\theta]_{255}$ −4400 for **6**, which were very similar to the maxima observed for sarasinoside A_2 (**2**) (Table I). Acidic hydrolysis of sarasinosides B_2 (**7**) and C_2 (**6**) with 3% aqueous H_2SO_4 respectively provided the same sapogenol **12**. So, it was shown that sarasinosides B_2 (**7**) and C_2 (**6**) possessed the same aglycone **12** as sarasinoside A_2 (**2**).

Sarasinoside B_2 (**7**) and sarasinoside C_2 (**6**) moved with the same *Rf* values as sarasinoside B_1 (**5**) and C_1 (**4**), respectively, on ordinary TLC. The carbon signals due to the sugar moiety in the ^{13}C-NMR spectrum of **7** were superimposable on those of **5**, while the carbon signals due to the sugar moiety of **6** were superimposable on those of **4**. Thus, **7** and **6** were presumed to be the 7,9(11)-diene analogs of **5** and **4**, respectively. In order to verify this, **7** and **6** were each subjected to catalytic hydrogenation over 10% palladium–carbon to afford their tetrahydro derivatives **27** and **26**. The reduction products, **27** and **26**, thus obtained were shown to be identical with the reduction products prepared from sarasinosides B_1 (**5**) and C_1 (**4**) under the same reaction conditions. Consequently, the chemical structures of sarasinosides B_2 and C_2 have been determined to be **7** and **6**, respectively.

Sarasinosides B_3 (9) and C_3 (8) Sarasinoside B_3 (**9**) and sarasinoside C_3 (**8**) were obtained as fine crystals of mp 190—193 °C and 187—190 °C, respectively. Sarasinosides B_3 (**9**) and C_3 (**8**) showed UV absorption maxima at 243 nm (ε=21600) (for **9**) and at 244 nm (ε=25900) (for **8**) and negative CD maxima of $[\theta]_{231}$ −14800 (for **9**) and $[\theta]_{231}$ −15900 (for **8**), which were characteristically similar to those observed for sarasinoside A_3 (**3**). Acidic hydrolysis of **9** and **8** with 5% aqueous H_2SO_4–MeOH (1 : 1) furnished the same sapogenol **13**. Therefore, it was shown that the aglycone of sarasinosides B_3 (**9**) and C_3 (**8**) had the same structure as that of sarasinoside A_3 (**3**).

The mobilities of sarasinoside B_3 (**9**) and sarasinoside C_3 (**8**) on ordinary TLC were the same as those of sarasinosides B_1 (**5**) and C_1 (**4**), as observed among the foregoing sarasinoside congenors, *e.g.* A_1 (**1**), A_2 (**2**), and A_3 (**3**). The carbon signals assignable to the sugar moiety in the ^{13}C-NMR spectrum of sarasinoside B_3 (**9**) closely resembled those observed for sarasinoside B_1 (**5**), while the carbon signals due to the sugar moiety of sarasinoside C_3 (**8**) were shown to be superimposable on those of sarasinoside C_1 (**4**). These findings indicated that **9** and **8** were the 8,14-diene analogs of **5** and **4**, respectively. In order to substantiate this presumption, sarasinosides B_3 (**9**) and C_3 (**8**) were subjected to catalytic hydrogenation over 10% palladium–carbon to afford the tetrahydro derivatives **27** and **26**. The products, **27** and **26**, thus obtained were shown to be identical with the reduction products which were synthesized from sarasinoside B_1 (**5**) and C_1 (**4**), respectively, through the same reaction procedures. Based on the accumulated evidence, the structures of sarasinoside B_3 and C_3 have been determined to be **9** and **8**, respectively.

The structures of sarasinosides elucidated in this paper are characterized by a norlanostane-triterpenoid aglycone. As far as we know, this is the first example of naturally occurring norlanostane-triterpenoidal saponins. The nor-

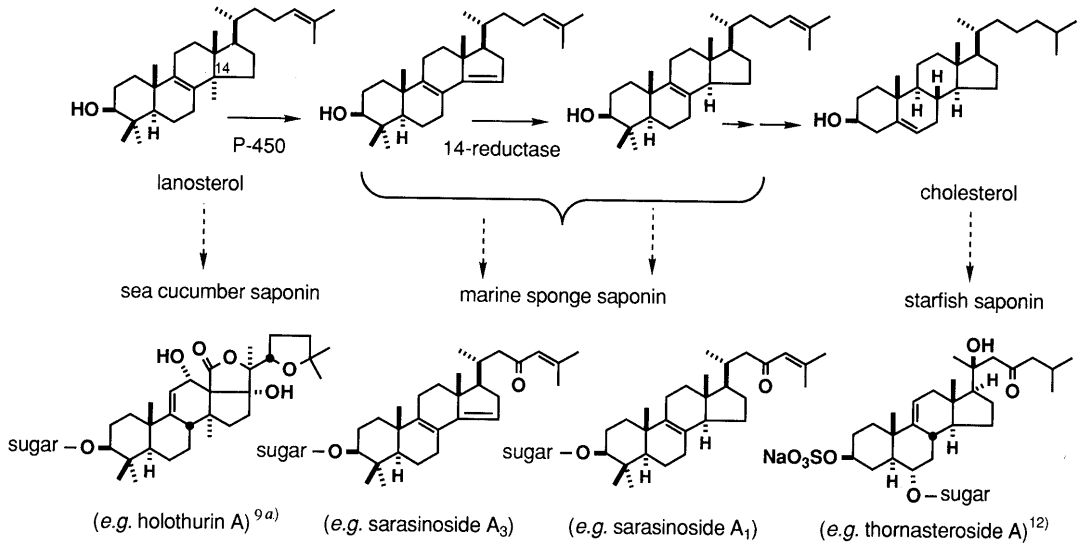


Fig. 1. Conversion of Lanosterol to 4,4-Dimethylzymosterol by Yeast Microsomes

lanostane-triterpenoid, the carbon skeleton of the aglycone of sarasinosides, has been placed as an intermediate in the biogenetic pathway from lanosterol to cholesterol in yeast and animal organs, where oxidative elimination of the 14α-methyl group has been demonstrated.[11] It is noteworthy from the viewpoint of chemosystematics that saponins from sea cucumbers possess lanostane-triterpenoidal aglycones[2,3,9] and those from starfish comprise steroidal aglycones,[12] while the saponins isolated in this paper from the marine sponge *Asteropus sarasinosum* have 30-norlanostane triterpenoids as their aglycones, although it remains to be proved that they are really biosynthesized by the respective parent organisms (Fig. 1).

Among the nine sarasinosides, the major saponins sarasinosides A_1 (**1**) and B_1 (**5**) have been subjected to some biological activity tests. It has been found so far that **1** and **5** exhibit piscicidal activity (against *Pocilia reticulata*), with LD_{50} (48 h) = 0.39 μg/ml for **1** and 0.71 μg/ml for **5**, and inhibit the cell division of fertilized eggs of the starfish *Asterina pectinifera* with LD_{100} = 10 μg/ml for **1** and **5**.

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in our preceding paper.[13]

Isolation of Sarasinosides A_1 (1), A_2 (2), A_3 (3), B_1 (5), B_2 (7), B_3 (9), C_1 (4), C_2 (6), and C_3 (8) Freeze-dried whole marine sponge (1.2 kg) *Asteropus sarasinosum* (collected in the Palau Islands), was extracted with 95% aqueous MeOH (20 l) at room temperature 3 times for 8 h each. The MeOH extract obtained after evaporation of the solvent *in vacuo* was partitioned into an ethyl acetate–H_2O mixture and then the H_2O phase was further partitioned with 1-butanol to furnish the 1-butanol-soluble portion (140 g after evaporation of the solvent). The 1-butanol-soluble portion (20 g) was then purified by column chromatography [Silica gel 60, 60—230 mesh (Merck), 2 kg, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)→6 : 4 : 1] to give three oligoglycoside fractions [sarasinoside A (1.4 g), sarasinoside B (1.1 g), and sarasinoside C (0.3 g)]. The oligoglycoside fractions were further subjected to HPLC (Zorbax ODS, MeOH : H_2O = 5 : 1) to isolate nine oligoglycosides: sarasinosides A_1 (**1**) (136 mg), A_2 (**2**) (49 mg), and A_3 (**3**) (80 mg) from the sarasinoside A fraction, sarasinosides B_1 (**5**) (100 mg), B_2 (**7**) (33 mg), and B_3 (**9**) (67 mg) from the sarasinoside B fraction, and sarasinosides C_1 (**4**) (26 mg), C_2 (**6**) (7 mg), C_3 (**8**) (20 mg) from the sarasinoside C fraction, respectively.

Sarasinoside A_1 (**1**): mp 207—210 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −14° (c = 1, MeOH, 25 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3370 (br), 2929, 2870, 1640 (br), 1550, 1067 (br). CD ($c = 1.0 \times 10^{-1}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.60 (3H, s), 1.00 (3H, d, J = 5.8 Hz, 20-CH_3), 1.01, 1.16, 1.29 (each 3H, s), 1.75 (3H, s, 25-CH_3), 2.04, 2.09 (both 3H, s, NHCOCH_3), 2.15 (3H, s, 25-CH_3), 3.12 (1H, dd, J = 4.4, 11.1 Hz, 3α-H), 4.53 (1H, d, J = 8.2 Hz), 5.15 (1H, d, J = 8.6 Hz), 5.20 (1H, d, J = 7.6 Hz), 5.56 (1H, d, J = 8.5 Hz), 5.58 (1H, d, J = 8.6 Hz), 6.14 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 172.0, 171.5 (both s, NHCOCH_3), 153.7 (s, C-25), 136.4 (s, C-9), 127.5 (s, C-8), 124.8 (d, C-24), 106.7 (d), 105.5 (d), 102.6 (d), 102.2 (d), 101.7 (d), 90.1 (d, C-3). *Anal.* Calcd for $C_{62}H_{100}N_2O_{26} \cdot 2H_2O$: C, 56.18; H, 7.91; N, 2.11. Found: C, 56.36; H, 7.79; N, 2.16.

Sarasinoside A_2 (**2**): mp 205—208 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −5.5° (c = 0.5, MeOH, 23 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3360 (br), 2915, 2873, 1630 (br), 1548, 1050 (br). CD ($c = 5.8 \times 10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.54 (3H, s), 0.99 (3H, d, J = 4.6 Hz, 20-CH_3), 1.03, 1.25, 1.29 (each 3H, s), 1.74 (3H, s, 25-CH_3), 2.03, 2.09 (both 3H, s, NHCOCH_3), 2.16 (3H, s, 25-CH_3), 3.15 (1H, dd, J = 3.7, 11.3 Hz, 3α-H), 4.55 (1H, d, J = 7.6 Hz), 5.18 (1H, d, J = 7.9 Hz), 5.20 (1H, d, J = 9.2 Hz), 5.53 (1H, d, J = 7.9 Hz), 5.55 (1H, d, J = 8.5 Hz), 5.33 (1H, m), 5.44 (1H, m), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.6 (s, C-23), 172.0, 171.5 (both s, NHCOCH_3), 153.9 (s, C-25), 147.3 (s, C-9), 135.8 (s, C-8), 124.9 (d, C-24), 122.0 (d, C-7), 117.2 (d, C-11), 106.8 (d), 105.6 (d), 102.8 (d), 102.4 (d), 101.9 (d), 90.1 (d, C-3). *Anal.* Calcd for $C_{62}H_{98}N_2O_{26} \cdot 2H_2O$: C, 56.27; H, 7.77; N, 2.12. Found: C, 56.20; H, 8.00; N, 1.94.

Sarasinoside A_3 (**3**): mp 205—208 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −22° (c = 1.2, MeOH, 23 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3290 (br), 2902, 2869, 1630 (br), 1549, 1050 (br). CD ($c = 5.5 \times 10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.85 (3H, s), 1.02 (3H, d, J = 6.4 Hz, 20-CH_3), 1.07, 1.17, 1.30 (each 3H, s), 1.75 (3H, s, 25-CH_3), 2.04, 2.09 (both 3H, s, NHCOCH_3), 2.16 (3H, s, 25-CH_3), 3.13 (1H, dd, J = 4.4, 11.5 Hz, 3α-H), 4.57 (1H, d, J = 7.6 Hz), 5.19 (1H, d, J = 8.6 Hz), 5.22 (1H, d, J = 7.6 Hz), 5.59 (1H, d, J = 8.9 Hz), 5.61 (1H, d, J = 8.9 Hz), 5.37 (1H, br s, 15-H), 6.16 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 171.9, 171.5 (both s, NHCOCH_3), 153.9 (s, C-25), 151.4 (s, C-14), 142.3 (s, C-9), 124.9 (d, C-24), 122.8 (s, C-8), 116.9 (d, C-15), 106.7 (d), 105.6 (d), 102.7 (d), 102.2 (d), 101.8 (d), 89.9 (d, C-3). *Anal.* Calcd for $C_{62}H_{98}N_2O_{26} \cdot 2H_2O$: C, 56.27; H, 7.77; N, 2.12. Found: C, 56.13; H, 7.93; N, 2.10.

Sarasinoside B_1 (**5**): mp 197—199 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −16° (c = 0.99, MeOH, 20 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3340 (br), 2933, 2871, 1640 (br), 1554, 1063 (br). CD ($c = 5.6 \times 10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.62, 0.98 (both 3H, s), 1.01 (3H, d, J = 5.5 Hz, 20-CH_3), 1.16, 1.32 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.04, 2.08 (both 3H, s, NHCOCH_3), 2.16 (3H, s, 25-CH_3), 3.15 (1H, dd, J = 4.3, 11.9 Hz, 3α-H), 4.57 (1H, d, J = 7.6 Hz), 5.20 (1H, d, J = 8.2 Hz), 5.21 (1H, d, J = 7.3 Hz), 5.43 (1H, d, J = 7.3 Hz), 5.56 (1H, d, J = 8.5 Hz), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.7 (s, C-23), 172.0, 171.5 (both s, NHCOCH_3), 153.8 (s, C-25), 136.4 (s, C-9), 127.7 (s, C-8), 124.9 (d, C-24),

106.7 (d), 105.5 (d), 103.2 (d), 102.3 (d), 102.0 (d), 90.0 (d, C-3). *Anal.* Calcd for $C_{61}H_{98}N_2O_{25}\cdot 2H_2O$: C, 56.56; H, 7.94; N, 2.16. Found: C, 56.61; H, 7.88; N, 2.32.

Sarasinoside B_2 (**7**): mp 192—195 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −10° ($c=0.3$, MeOH). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3370 (br), 2912, 2866, 1640 (br), 1542, 1065 (br). CD ($c=1.3\times10^{-1}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine−D_2O) δ: 0.54 (3H, s), 1.00 (6H, br s), 1.23, 1.31 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.03, 2.08 (both 3H, s, NHCOCH_3), 2.17 (3H, s, 25-CH_3), 3.16 (1H, dd, $J=4.1$, 11.8 Hz, 3α-H), 4.58 (1H, d, $J=7.3$ Hz), 5.20 (1H, d, $J=7.3$ Hz), 5.23 (1H, d, $J=8.5$ Hz), 5.38 (1H, d, $J=7.3$ Hz), 5.54 (1H, d, $J=8.5$ Hz), 5.35 (1H, m), 5.44 (1H, m), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 172.0, 171.4 (both s, NHCOCH_3), 153.8 (s, C-25), 147.2 (s, C-9), 135.8 (s, C-8), 124.9 (d, C-24), 121.9 (d, C-7), 117.2 (d, C-11), 106.7 (d), 105.3 (d), 103.2 (d), 102.3 (d), 102.0 (d), 89.8 (d, C-3). *Anal.* Calcd for $C_{61}H_{96}N_2O_{25}$: C, 58.27; H, 7.70; N, 2.23. Found: C, 58.12; H, 8.00; N, 2.28.

Sarasinoside B_3 (**9**): mp 190—193 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −23° ($c=0.4$, MeOH, 25 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3360 (br), 2919, 2875, 1640 (br), 1547, 1071 (br). CD ($c=8.3\times10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.87, 1.03 (both 3H, s), 1.03 (3H, d, $J=5.8$ Hz, 20-CH_3), 1.16, 1.31 (both 3H, s), 1.75 (3H, s, 25-CH_3), 2.04, 2.08 (both 3H, s, NHCOCH_3), 2.18 (3H, s, 25-CH_3), 3.15 (1H, dd, $J=4.3$, 11.9 Hz, 3α-H), 4.58 (1H, d, $J=7.3$ Hz), 5.22 (2H, d, $J=7.9$ Hz), 5.49 (1H, d, $J=7.6$ Hz), 5.57 (1H, d, $J=8.2$ Hz), 5.37 (1H, br s, 15-H), 6.16 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 171.9, 171.5 (both s, NHCOCH_3), 153.8 (s, C-25), 151.2 (s, C-14), 142.1 (s, C-9), 124.8 (d, C-24), 122.8 (s, C-8), 116.9 (d, C-15), 106.4 (d), 105.3 (d), 103.0 (d), 102.1 (d), 101.8 (d), 89.6 (d, C-3). *Anal.* Calcd for $C_{61}H_{96}N_2O_{25}$: C, 58.27; H, 7.70; N, 2.23. Found: C, 58.29; H, 8.01; N, 2.34.

Sarasinoside C_1 (**4**): mp 194—197 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −22° ($c=2.65$, MeOH, 25 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3320 (br), 2933, 2860, 1637 (br), 1546, 1043 (br). CD ($c=6.7\times10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.60, 0.94 (both 3H, s), 1.01 (3H, d, $J=5.5$ Hz, 20-CH_3), 1.12, 1.27 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.09, 2.10 (both 3H, s, NHCOCH_3), 2.15 (3H, s, 25-CH_3), 3.18 (1H, dd, $J=4.3$, 11.9 Hz, 3α-H), 4.62 (1H, d, $J=7.3$ Hz), 4.94 (1H, d, $J=7.6$ Hz), 5.09 (1H, d, $J=8.6$ Hz), 5.43 (1H, d, $J=8.5$ Hz), 6.14 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 172.1, 171.7 (both s, NHCOCH_3), 153.7 (s, C-25), 135.9 (s, C-9), 127.3 (s, C-8), 124.5 (d, C-24), 104.6 (d), 104.3 (d), 101.6 (2C, d), 89.0 (d, C-3). *Anal.* Calcd for $C_{55}H_{88}N_2O_{20}\cdot 2H_2O$: C, 58.29; H, 8.18; N, 2.47. Found: C, 58.02, H, 7.88; N, 2.44.

Sarasinoside C_2 (**6**): mp 188—191 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −11° ($c=0.3$, MeOH, 25 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3370 (br), 2930, 2873, 1640 (br), 1547, 1050 (br). CD ($c=7.5\times10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.53, 0.95 (both 3H, s), 0.98 (3H, d, $J=6.1$ Hz, 20-CH_3), 1.19, 1.26 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.08, 2.10 (both 3H, s, NHCOCH_3), 2.15 (3H, s, 25-CH_3), 3.18 (1H, dd, $J=3.8$, 12.1 Hz, 3α-H), 4.60 (1H, d, $J=7.3$ Hz), 4.94 (1H, d, $J=7.3$ Hz), 5.10 (1H, d, $J=8.2$ Hz), 5.39 (1H, d, $J=8.5$ Hz), 5.35 (1H, m), 5.41 (1H, m), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.5 (s, C-23), 172.3, 171.6 (both s, NHCOCH_3), 153.8 (s, C-25), 147.0 (s, C-9), 135.8 (s, C-8), 124.7 (d, C-24), 121.7 (d, C-7), 117.0 (d, C-11), 105.0 (d), 104.5 (d), 102.0 (2C, d), 89.1 (d, C-3). *Anal.* Calcd for $C_{55}H_{86}N_2O_{20}\cdot 2H_2O$: C, 58.39; H, 8.02; N, 2.48. Found: C, 58.65; H, 8.16; N, 2.44.

Sarasinoside C_3 (**8**): mp 187—190 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −31° ($c=0.9$, MeOH, 25 °C). UV (MeOH) λ_{max}: Table I. IR (KBr) ν_{max} cm^{-1}: 3380 (br), 2913, 2865, 1630 (br), 1543, 1040 (br). ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.85, 0.98 (both 3H, s), 1.03 (3H, d, $J=6.1$ Hz, 20-CH_3), 1.12, 1.27 (both 3H, s), 1.75 (3H, s, 25-CH_3), 2.08, 2.09 (both 3H, s, NHCOCH_3), 2.16 (3H, s, 25-CH_3), 3.17 (1H, dd, $J=4.0$, 11.6 Hz, 3α-H), 4.63 (1H, d, $J=7.0$ Hz), 4.95 (1H, d, $J=7.3$ Hz), 5.11 (1H, d, $J=8.2$ Hz), 5.43 (1H, d, $J=8.2$ Hz), 5.37 (1H, br s, 15-H), 6.16 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.4 (s, C-23), 172.7, 171.8 (both s, NHCOCH_3), 153.8 (s, C-25), 151.1 (s, C-14), 141.9 (s, C-9), 124.7 (d, C-24), 122.7 (s, C-8), 116.7 (d, C-15), 104.8 (d), 104.4 (d), 101.8 (2C, d), 88.8 (d, C-3). *Anal.* Calcd for $C_{55}H_{86}N_2O_{20}\cdot H_2O$: C, 59.34; H, 7.97; N, 2.52. Found: C, 59.16; H, 8.16; N, 2.51.

Acidic Hydrolysis of Sarasinosides A_1 (1), B_1 (5), and C_1 (4) A mixture of **1** (60 mg) and 20% aqueous H_2SO_4–MeOH (1 : 1) (2 ml) was heated under reflux for 3 h on a water-bath. After dilution with H_2O, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (20 mg), which was purified by column chromatography (SiO_2 treated with $AgNO_3$, benzene : acetone = 30 : 1) to furnish the sapogenols, **10** (9 mg) and **11** (5 mg). **10**: mp 124—127 °C (MeOH–AcOEt), colorless needles. $[\alpha]_D$ +5.2° ($c=0.5$, $CHCl_3$, 20 °C). UV (MeOH) λ_{max} nm (ε): 236 (12200). IR (CCl_4) ν_{max} cm^{-1}: 3628, 2938, 2872, 1690, 1621, 1448, 1029. CD ($c=1.2\times10^{-2}$, MeOH): $[\theta]_{223}$ −7600 (neg. max.), $[\theta]_{203}$ +5500 (pos. max.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.75, 0.81, 0.89 (each 3H, s), 0.95 (3H, d, $J=6.1$ Hz, 20-CH_3), 1.01 (3H, s), 1.88, 2.14 (both 3H, s, 25-CH_3), 3.26 (1H, dd, $J=4.1$, 11.8 Hz, 3α-H), 6.06 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.6 (s, C-23), 153.9 (s, C-25), 141.8 (s, C-14), 127.1 (s, C-8), 125.0 (d, C-24), 78.4 (d, C-3). Mass *m/z* (%): 426 (8, M^+), 408 (3), 83 (100). High-resolution Mass Calcd for $C_{29}H_{46}O_2$: 426.350. Found: 426.349. **11**: mp 153—155 °C (MeOH–AcOEt), colorless needles. $[\alpha]_D$ +12.2° ($c=0.18$, $CHCl_3$, 20 °C). UV (MeOH) λ_{max} nm (ε): 239 (13300). IR (CCl_4) ν_{max} cm^{-1}: 3622, 2930, 2850, 1688, 1618, 1446, 1031. CD ($c=1.77\times10^{-2}$, MeOH): $[\theta]_{242}$ −1400 (neg. max.), $[\theta]_{210}$ −1900 (neg. max.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81, 0.89 (both 3H, s), 0.94 (3H, d, $J=6.4$ Hz, 20-CH_3), 0.95, 0.98 (both 3H, s), 1.89, 2.15 (both 3H, s, 25-CH_3), 3.21 (1H, dd, $J=4.6$, 11.6 Hz, 3α-H), 5.13 (1H, m, 15-H), 6.07 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.6 (s, C-23), 155.8 (s, C-14), 153.9 (s, C-25), 125.0 (d, C-24), 117.2 (d, C-15), 78.3 (d, C-3). Mass *m/z* (%): 426 (4, M^+), 408 (3), 125 (100). High-resolution Mass Calcd for $C_{29}H_{46}O_2$: 426.350. Found : 426.349.

Sarasinosides B_1 (**5**) (20 mg) and C_1 (**4**) (15 mg) were hydrolyzed as described above to give the sapogenols, **10** and **11**, respectively. Compounds **10** and **11** thus obtained were shown to be identical with authentic samples obtained above from **1** by TLC and mass spectral comparisons. Furthermore, a mixture of **1** (23 mg) and 10% aqueous H_2SO_4–MeOH (1 : 1) (2 ml) was heated under reflux for 2 h on a water-bath. The reaction mixture was worked up as described above to give **10** (3 mg) as a single product.

Enzymatic Hydrolysis of Sarasinoside A_1 (1) a) A suspension of **1** (350 mg) in H_2O (5 ml) was treated with crude hesperidinase (500 mg, lot No. 680930 provided by Tanabe Pharm. Co.) and the whole mixture was stirred at 40 °C for 4 d. The reaction mixture was then treated with 1-butanol (5 ml), heated at 60 °C for 10 min and filtered. The filtrate was extracted with 1-butanol and the 1-butanol extract was washed with H_2O and concentrated under reduced pressure to give a product (280 mg). Purification of the product by column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)] furnished A_1-pro-1 (**15**) (30 mg) and A_1-pro-2 (**16**) (62 mg). A_1-pro-1 (**15**): mp 171—174 °C (80% MeOH), fine crystals. $[\alpha]_D$ −20° ($c=0.3$, MeOH, 20 °C). UV (MeOH) λ_{max} nm (ε): 238 (12200). IR (KBr) ν_{max} cm^{-1}: 3310 (br), 2935, 2865, 1640 (br), 1544, 1071 (br). CD ($c=2.74\times10^{-2}$, MeOH): $[\theta]_{222}$ −5600 (neg. max). ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.59, 0.89 (both 3H, s), 1.00 (3H, d, $J=5.8$ Hz, 20-CH_3), 1.06, 1.27 (both 3H, s), 1.75 (3H, s, 25-CH_3), 2.09 (3H, s, NHCOCH_3), 2.14 (3H, s, 25-CH_3), 3.22 (1H, dd, $J=4.1$, 11.9 Hz, 3α-H), 4.76 (1H, d, $J=7.0$ Hz), 5.62 (1H, d, $J=8.5$ Hz), 6.14 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.9 (s, C-23), 172.0 (s, NHCOCH_3), 154.0 (s, C-25), 135.9 (s, C-9), 127.3 (s, C-8), 124.5 (d, C-24), 104.6 (d), 101.7 (d), 89.2 (d, C-3), 62.5 (t, C-6″). *Anal.* Calcd for $C_{42}H_{67}NO_{11}\cdot 2H_2O$: C, 63.21; H, 8.46; N, 1.76. Found: C, 63.55; H, 8.73; N, 1.75. A_1-pro-2 (**16**): mp 196—198 °C (80% MeOH), fine crystals. $[\alpha]_D$ −13° ($c=0.26$, MeOH, 20 °C). UV (MeOH) λ_{max} nm (ε): 239 (13800). IR (KBr) ν_{max} cm^{-1}: 3360 (br), 2941, 2875, 1655 (br), 1549, 1069 (br). CD ($c=4.05\times10^{-2}$, MeOH): $[\theta]_{222}$ −7200 (neg. max.). ^{1}H-NMR (500 MHz, d_5-pyridine+D_2O) δ: 0.60, 0.94 (both 3H, s), 1.01 (3H, d, $J=5.8$ Hz, 20-CH_3), 1.13, 1.27 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.09, 2.10 (both 3H, s, NHCOCH_3), 2.15 (3H, s, 25-CH_3), 3.17 (1H, dd, $J=4.3$, 11.9 Hz, 3α-H), 4.61 (1H, d, $J=7.3$ Hz), 5.05 (1H, d, $J=7.6$ Hz), 5.10 (1H, d, $J=8.2$ Hz), 5.46 (1H, d, $J=8.5$ Hz), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.7 (s, C-23), 172.2, 171.7 (both s, NHCOCH_3), 153.8 (s, C-25), 135.8 (s, C-9), 127.5 (s, C-8), 124.7 (d, C-24), 104.6 (d), 104.5 (d), 101.9 (d), 101.6 (d), 89.3 (d, C-3), 69.8 (t, C-6″). *Anal.* Calcd for $C_{56}H_{90}N_2O_{21}\cdot 3H_2O$: C, 56.93; H, 8.19; N, 2.37. Found: C, 56.68; H, 7.87; N, 2.43.

b) A suspension of **1** (200 mg) in H_2O (5 ml) was treated with β-glucosidase (type II, from almonds) (400 mg) and the whole was kept stirring at 40 °C for 6 d. The 1-butanol extractive (170 mg), which was obtained by working up of the reaction mixture as for the above-described hydrolysis with crude hesperidinase, was subjected to column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to furnish A_1-pro-3 (**17**) (20 mg) and unchanged **1** (80 mg). A_1-pro-3 (**17**): mp

189—192 °C (80% MeOH), fine crystals. $[\alpha]_D$ −25° (c = 0.2, MeOH, 20 °C). UV (MeOH) λ_{max} nm (ε): 237 (13400). IR (KBr) ν_{max} cm^{-1}: 3390 (br), 2936, 2847, 1629 (br), 1555, 1077 (br). CD ($c = 4.3 \times 10^{-2}$, MeOH): $[\theta]_{223}$ −5500 (neg. max.). ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.65 (3H, s), 1.05 (3H, d, J = 6.3 Hz, 20-CH_3), 1.07, 1.24, 1.34 (each 3H, s), 1.75 (3H, s, 25-CH_3), 1.95 (3H, s, NHCOCH_3), 2.21 (3H, s, 25-CH_3), 3.25 (1H, dd, J = 4.3, 11.9 Hz, 3α-H), 4.78 (1H, d, J = 7.3 Hz), 5.28 (1H, d, J = 7.0 Hz), 5.53 (1H, d, J = 7.3 Hz), 5.79 (1H, d, J = 6.7 Hz), 6.16 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.8 (s, C-23), 171.8 (s, NHCOCH_3), 153.8 (s, C-25), 135.8 (s, C-9), 127.5 (s, C-8), 124.8 (d, C-24), 106.3 (d), 105.6 (d), 102.8 (d), 101.5 (d), 90.0 (d, C-3), 69.6 (t, C-6″). *Anal.* Calcd for $C_{54}H_{87}NO_{21} \cdot 2H_2O$: C, 57.79; H, 7.99; N, 1.25. Found: C, 57.94; H, 8.18; N, 1.26.

Carbohydrate Composition of Sarasinoside A_1 (1) and Prosapogenols (15, 16, 17) A solution of **1**, **15**, **16**, or **17** (3 mg each) in anhydrous 9% HCl–MeOH (0.5 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated under reduced pressure to give the product, which was trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) in pyridine (0.1 ml), and the resulting trimethylsilyl derivatives were quantitatively analyzed by GLC 2% silicone SE-30 on Uniport B 80—100 mesh; 3 mm × 2 m; column temperature, 180 °C; N_2 flow rate, 35 ml/min) to identify methyl xylopyranoside (a) (t_R = 2 min 57 s, 3 min 11 s), methyl glucopyranoside (b) (t_R = 7 min 07 s, 7 min 47 s), methyl 2-acetamide-2-deoxy-galactopyranoside (c) (t_R = 16 min 02 s), and methyl 2-acetamide-2-deoxy-glucopyranoside (d) (t_R = 17 min 54 s). As standards, 10 mg each of xylose, glucose, *N*-acetylglucosamine, and *N*-acetylgalactosamine were treated in a similar manner. The relative integrated areas (given in parentheses) of the GLC peaks were as follows: **1**: a (1), b (2), c (1), d (1); **15**: a (1), d (1); **16**: a (1), b (1), c (1), d (1); **17**: a (1), b (2), d (1). A solution of sarasinosides mixture (1.2 g) in anhydrous 9% HCl–MeOH (10 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated under reduced pressure to give the product (1.4 g), which was separated by column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)] to furnish methyl glucopyranoside (213 mg), methyl xylopyranoside (199 mg) and a mixture of methyl aminoglycosides (286 mg). The mixture of methyl aminoglycosides (286 mg) was further separated by HPLC (Zorbax ODS, MeOH : H_2O = 1 : 10) to give methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (78 mg) and methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (45 mg), which were identical with authentic samples by ^{1}H-NMR, HPLC, TLC, and $[\alpha]_D$ comparisons. Both methyl glucopyranoside (90 mg) and methyl xylopyranoside (126 mg) obtained above were hydrolyzed with 5% aqueous HCl (3 ml) under reflux for 1 h, respectively. The reaction mixtures were neutralized with Dowex 1 × 2 OH^- form and filtered. After removal of the solvent, both products were purified by column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to give D-glucose (20 mg) and D-xylose (30 mg), which were identical with the respective authentic samples by ^{1}H-NMR, TLC, and $[\alpha]_D$ comparisons.

Methylation of A_1-pro-1 (15) Followed by Methanolysis 1) A solution of **15** (18 mg) in dimethyl sulfoxide (DMSO) (1 ml) was treated with a dimsyl carbanion solution (1 ml) [prepared from NaH (2 g), which was washed with dry *n*-hexane before use, and DMSO (35 ml) by stirring at 60 °C for 1 h under an N_2 atmosphere] and the whole solution was sirred at room temperature (25 °C) for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (1 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. The reaction mixture was then poured into ice-H_2O and the whole was extracted with AcOEt. The AcOEt extract was washed with H_2O, then dried over $MgSO_4$. Removal of the solvent from the AcOEt extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2, benzene : acetone = 3 : 1) to furnish the fully methylated derivative (10 mg); IR (CCl_4): no OH.

2) A solution of the fully methylated derivative (4 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1 h and neutralized with Ag_2CO_3. The whole mixture was filtered and the filtrate was subjected to GLC analysis [1) 15% polyethylene glycol succinate (PEGS) on Chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temperature, 190 °C; N_2 flow rate, 35 ml/min, and 2) 5% butane disuccinate (BDS) on Uniport B (80—100 mesh); 3 mm × 2 m; column temperature, 165 °C; N_2 flow rare, 35 ml/min] and TLC (benzene–acetone = 3 : 1). Methyl 3,4-di-*O*-methylxylopyranoside [I] was identified [1) PEGS t_R = 4 min 55 s, 5 min 59 s; 2) BDS t_R = 4 min 50 s, 5 min 53 s; Rf = 0.32].

Methylation of A_1-pro-2 (16) Followed by Methanolysis 1) A solution of **16** (20 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (2 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (1 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work up of the reaction mixture as described above gave a product, which was purified by column chromatography (SiO_2, benzene : acetone = 3 : 1) to furnish the fully methylated derivative (13 mg); IR (CCl_4): no OH.

2) A solution of the fully methylated derivative (5 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was examined by GLC and TLC (benzene : acetone = 1 : 1). The following methyl glycosides were identified: methyl 3-*O*-methylxylopyranoside [II] [1) PEGS t_R = 12 min 52 s, 20 min 02 s; 2) BDS t_R = 11 min 49 s; Rf = 0.34] and methyl 2,3,4,6-tetra-*O*-methylglucopyranoside [III] [1) PEGS t_R = 3 min 15 s, 4 min 27 s; 2) BDS t_R = 3 min 41 s, 5 min 11 s; Rf = 0.75, 0.80].

Methylation of A_1-pro-3 (17) Followed by Methanolysis 1) A solution of **17** (15 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (2 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (2 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work-up of the reaction mixture as described above gave a product, which was purified by column chromatography (SiO_2, benzene : acetone = 3 : 1) to furnish the fully methylated derivative (7 mg); IR (CCl_4): no OH.

2) A solution of the fully methylated derivative (4 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1.5 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was analyzed by GLC and TLC (benzene : acetone = 3 : 1) to identify [I] [1) PEGS t_R = 4 min 58 s, 6 min 03 s; 2) BDS t_R = 4 min 37 s, 5 min 32 s; Rf = 0.34], [III] [1) PEGS t_R = 3 min 22 s, 4 min 36 s; 2) BDS t_R = 3 min 45 s, 5 min 18 s; Rf = 0.50, 0.60], and methyl 3,4,6-tri-*O*-methylglucopyranoside [IV] [1) PEGS t_R = 9 min 50 s, 11 min 54 s; 2) BDS t_R = 10 min 47 s, 13 min 07 s; Rf = 0.26].

Methylation of Sarasinoside A_1 (1) Followed by Methanolysis 1) A solution of **1** (20 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (2 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (2 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work-up of the reaction mixture as described above gave a product, which was purified by column chromatography (SiO_2, benzene : acetone = 1 : 1) to furnish the fully methylated derivative (10 mg); IR (CCl_4) ν_{max} cm^{-1}: no OH, 2926, 1707, 1648, 1057 (br).

2) A solution of the fully methylated derivative (4 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1.5 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was analyzed by GLC [3) 15% neopentyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temperature, 190 °C; N_2 flow rate 35 ml/min] and TLC (benzene : acetone = 1 : 1) to identify [II] [1) PEGS t_R = 13 min 04 s, 20 min 08 s; 3) NPGS t_R = 13 min 20 s, 19 min 22 s; Rf = 0.35], [III] [1) PEGS t_R = 3 min 15 s, 4 min 26 s; 3) NPGS t_R = 5 min 20 s, 7 min 06 s; Rf = 0.72, 0.79], [IV] [1) PEGS t_R = 9 min 23 s, 11 min 26 s; 3) NPGS t_R = 12 min 48 s, 14 min 46 s; Rf = 0.50].

Enzymatic Hydrolysis of Sarasinoside B_1 (5) a) A suspension of **5** (170 mg) in H_2O (4 ml) was treated with crude hesperidinase (500 mg, lot NO. 680930 provided by Tanabe Pharm. Co.) and the whole mixture was stirred at 40 °C for 12 d. The reaction mixture was then treated with 1-butanol (5 ml), heated at 60 °C for 10 min, and filtered. The filtrate was extracted with 1-butanol and the 1-butanol extract was washed with H_2O and concentrated under reduced pressure to give a product (140 mg). Purification of the product by column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)] furnished B_1-pro-1 (**15**) (10 mg) and B_1-pro-2 (**4**) (37 mg). B_1-pro-1 and B_1-pro-2 were shown to be identical with A_1-pro-1 (**15**) and sarasinoside C_1 (**4**), respectively, by mixed melting-point determination and UV, IR, ^{13}C-NMR, HPLC, and TLC comparisons.

b) A suspension of **5** (50 mg) in H_2O (2 ml) was treated with β-glucosidase (type II, from almonds) (200 mg) and the whole was kept stirring at 38 °C for 12 d. The 1-butanol extractive (40 mg), which was obtained by work-up of the reaction mixture as for the above-described hydrolysis with crude hesperidinase, was subjected to column chromatography [SiO_2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to furnish B_1-pro-3 (**18**) (19 mg) and unchanged **5** (12 mg). B_1-pro-3 (**18**): mp 188—191 °C (80% MeOH), fine crystals. $[\alpha]_D$ −20° (c = 0.24, MeOH, 20 °C). UV (MeOH) λ_{max} nm (ε): 238 (13700). IR (KBr) ν_{max} cm^{-1}: 3410 (br), 2920, 2850, 1628 (br), 1561, 1076 (br). CD ($c = 6.2 \times 10^{-2}$, MeOH): $[\theta]_{223}$ −5800 (neg. max.). ^{1}H-NMR

(500 MHz, d_5-pyridine + D_2O) δ: 0.61, 0.97 (both 3H, s), 1.01 (3H, d, J=5.8 Hz, 20-CH_3), 1.16, 1.34 (both 3H, s), 1.74 (3H, s, 25-CH_3), 2.01 (3H, s, NHCOCH_3), 2.17 (3H, s, 25-CH_3), 3.19 (1H, dd, J=4.3, 11.9 Hz, 3α-H), 4.70 (1H, d, J=7.3 Hz), 5.23 (1H, d, J=6.7 Hz), 5.27 (1H, d, J=7.3 Hz), 5.70 (1H, d, J=8.2 Hz), 6.15 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.9 (s, C-23), 171.9 (s, NHCOCH_3), 153.9 (s, C-25), 135.8 (s, C-9), 127.5 (s, C-8), 124.7 (d, C-24), 106.0 (d), 105.3 (d), 103.0 (d), 101.5 (d), 89.7 (d, C-3), 69.2 (t, C-6″). *Anal.* Calcd for $C_{53}H_{85}NO_{20}\cdot 2H_2O$: C, 58.28; H, 8.21; N, 1.28. Found: C, 58.22; H, 8.12; N, 1.27.

Carbohydrate Composition of Sarasinoside B_1 (5) and Prosapogenols (4, 18) A solution of **5**, **4**, or **18** (4 mg each) in anhydrous 9% HCl–MeOH (1 ml) was heated under reflux for 1 h. The reaction mixture was worked up and analyzed by GLC as described above to obtain the following results. Sarasinoside B_1 (**5**): a (2), b (1), c (1), d (1); **4**: a (2), c (1), d (1); **18**: a (2), b (1), d (1).

Methylation of B_1-pro-2 (4) Followed by Methanolysis 1) A solution of **4** (35 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (1 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (1 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work up of the reaction mixture as described above gave an AcOEt extract, which was purified by column chromatography (SiO_2, benzene : acetone = 2 : 1) to furnish the fully methylated derivative (19 mg); IR (CCl_4): no OH.

2) A solution of the fully methylated derivative (5 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was examined by GLC and TLC (benzene : acetone = 1 : 1). The following methyl glycosides were identified: [II] [1) PEGS t_R = 12 min 43 s, 19 min 34 s; 2) BDS t_R = 11 min 38 s; Rf = 0.34] and methyl 2,3,4-tri-*O*-methylxylopyranoside [V] [1) PEGS t_R = 1 min 45 s, 2 min 11 s; 2) BDS t_R = 1 min 46 s, 2 min 10 s; Rf = 0.76, 0.84].

Methylation of B_1-pro-3 (18) Followed by Methanolysis 1) A solution of **18** (15 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (2 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (2 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work-up of the reaction mixture as described above gave an AcOEt extract, which was purified by column chromatography (SiO_2, benzene : acetone = 3 : 1) to furnish the fully methylated derivative (7 mg); IR (CCl_4): no OH.

2) A solution of the fully methylated derivative (4 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was examined by GLC and TLC (benzene : acetone = 3 : 1). The following methyl glycosides were identified: [I] [1) PEGS t_R = 4 min 55 s, 5 min 58 s; 2) BDS t_R = 4 min 49 s, 5 min 47 s; Rf = 0.32] and [III] [1) PEGS t_R = 3 min 20 s, 4 min 32 s; 2) BDS t_R = 3 min 52 s, 5 min 31 s; Rf = 0.52, 0.61].

Methylation of Sarasinoside B_1 (5) Followed by Methanolysis 1) A solution of **5** (24 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (3 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (3 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. Work-up of the reaction mixture as described above gave an AcOEt extract, which was purified by column chromatography (SiO_2, benzene : acetone = 1 : 1) to furnish the fully methylated derivative (10 mg), IR (CCl_4) ν_{max} cm^{-1}: no OH, 2926, 1705, 1649, 1097 (br).

2) A solution of the fully methylated derivative (4 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was examined by GLC and TLC (benzene : acetone = 1 : 1). The following methyl glycosides were identified: [I] [1) PEGS t_R = 4 min 45 s, 5 min 44 s; 3) NPGS t_R = 6 min 07 s, 7 min 05 s; Rf = 0.59] [II] [1) PEGS t_R = 13 min 05 s, 20 min 08 s; 3) NPGS t_R = 13 min 25 s, 19 min 23 s; Rf = 0.34], and [III] [1) PEGS t_R = 3 min 18 s, 4 min 23 s; 3) NPGS t_R = 5 min 16 s, 7 min 05 s; Rf = 0.72, 0.80].

Complete Methylation of Sarasinoside A_1(1) Followed by Acetolysis, Hydrolysis and Derivation to Give Partially Methylated Hexitol Acetates 1) A solution of **1** (50 mg) in DMSO (2 ml) was treated with a dimsyl carbanion solution (4 ml) and the whole mixture was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (4 ml) under ice-cooling and stirred at room temperature for 1 h in the dark. The reaction mixture was then poured into ice-H_2O and the whole was extracted with AcOEt. The AcOEt extract was washed with H_2O, then dried over $MgSO_4$. Removal of the solvent from the AcOEt extract under reduced pressure gave a product, which was purified by SiO_2 column chromatography (benzene : acetone = 1 : 1) to furnish the fully methylated derivative (27 mg).

2) The fully methylated derivative (27 mg) was treated with 0.5 N H_2SO_4 in 95% acetic acid (1 ml) [prepared from 10 N H_2SO_4 (5 ml) and glacial acetic acid (95 ml)], and the whole was heated at 80 °C for 8 h. The reaction mixture was then treated with H_2O (1 ml) and heated at 80 °C for an additional 5 h.

3) The reaction mixture was neutralized with Dowex 1 × 2 OH^- form and the filtrate was evaporated to dryness under reduced pressure. A solution of the residue in MeOH–H_2O (1 : 1) (1 ml) was treated with sodium borohydride ($NaBH_4$) (40 mg) and was then stirred at room temperature for 6 h. The reaction mixture was treated with glacial acetic acid (0.5 ml) and the whole was evaporated under reduced pressure. The residue was treated with acetic anhydride (3 ml) and heated at 100 °C for 3 h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned into $CHCl_3$–H_2O. The $CHCl_3$-soluble portion was evaporated under reduced pressure to give the residue (35 mg), which was purifed by SiO_2 column chromatography (benzene : AcOEt = 3 : 1) to give 3-*O*-methyl-1,2,4,5-tetra-*O*-acetyl-xylitol (**19**, 3 mg), 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-glucitol (**20**, 3 mg), 3,4,6-tri-*O*-methyl-1,2,5-tri-*O*-acetyl-glucitol (**21**, 2 mg), 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidogalactitol (**23**, 3 mg), and 3,4-di-*O*-methyl-1,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol (**24**, 3 mg). 3-*O*-Methyl-1,2,4,5-tetra-*O*-acetyl-xylitol (**19**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.05, 2.11 (both 6H, s), 3.52 (3H, s), 3.58 (1H, dd, J=5.0, 5.0 Hz, 3-H), 4.08 (2H, dd, J=6.6, 12.0 Hz, 1-H, 5-H), 4.39 (2H, dd, J=4.0, 12.0 Hz, 1-H, 5-H), 5.25 (2H, ddd, J=4.0, 5.0, 6.6 Hz, 2-H, 4-H). Mass m/z (%): 275 (1, M^+ − OAc), 189 (54), 129 (100). High-resolution Mass Calcd for $C_{14}H_{22}O_9$ − OAc (M^+ − OAc): 275.113. Found: 275.112. 2,3,4,6-Tetra-*O*-methyl-1,5-di-*O*-acetyl-glucitol (**20**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.10, 2.12 (both 3H, s), 3.37, 3.50, 3.50, 3.51 (each 3H, s), 3.38 (1H, dd, J=4.0, 5.5 Hz, 3-H), 3.60 (1H, dd, J=4.0, 5.5 Hz, 4-H), 3.61 (1H, dd, J=5.5, 11.0 Hz, 6-H), 3.65 (1H, ddd, J=3.5, 5.5, 6.4 Hz, 2-H), 3.69 (1H, dd, J=3.4, 11.0 Hz, 6-H), 4.17 (1H, dd, J=6.4, 12.0 Hz, 1-H), 4.37 (1H, dd, J=3.5, 12.0 Hz, 1-H), 5.15 (1H, ddd, J=3.4, 5.5, 5.5 Hz, 5-H). Mass m/z (%): 291 (0.1, M^+ − OCH_3), 263 (M^+ − OAc, 1.3), 101 (100). High-resolution Mass Calcd for $C_{14}H_{26}O_8$ − OAc: 263.149. Found: 263.149. 3,4,6-Tri-*O*-methyl-1,2,5,-tri-*O*-acetyl-glucitol (**21**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.07, 2.10, 2.14, 3.37, 3.50, 3.52 (each 3H, s), 3.40 (1H, dd, J=5.2, 5.2 Hz, 3-H), 3.58 (1H, dd, J=4.9, 10.7 Hz, 6-H), 3.59 (1H, m, 4-H), 3.69 (1H, dd, J=4.0, 10.7 Hz, 6-H), 4.20 (1H, dd, J=7.5, 12.1 Hz, 1-H), 4.45 (1H, dd, J=3.2, 12.1 Hz, 1-H), 5.09 (1H, ddd, J=4.0, 4.9, 5.2 Hz, 5-H), 5.37 (1H, ddd, J=3.2, 5.2, 7.5 Hz, 2-H). Mass m/z (%): 291 (0.6, M^+ − OAc), 233 (1.4), 205 (2.6), 129 (100). High-resolution Mass Calcd for $C_{15}H_{26}O_9$ − OAc: 291.144. Found: 291.142. 3,4,6-Tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidogalactitol (**23**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.04[a], 2.06[b], 2.11[a] × 2, 2.12[b], 2.14[b] (total 9H, each s), 2.92[b], 3.02[a] (total 3H, both s), 3.30—3.48 (total 2H, 3-H, 4-H), 3.37[a] × 3, 3.40[b], 3.41[b], 3.43[b] (total 9H, each s), 3.49—3.60 (total 2H, 6-H), 4.17—4.38 (total 3H, 1-H, 2-H), 5.22 (total 1H, 5-H) (peak area a : b = 10 : 7). Mass m/z (%): 364 (2.8, M^+ + 1), 304 (1.1, M^+ − OAc), 116 (100). High-resolution Mass Calcd for $C_{16}H_{29}NO_8$: 363.189. Found: 363.189. 3,4-Di-*O*-methyl-1,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol (**24**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.05[a], 2.07, 2.08[a], 2.10[b], 2.12[a], 2.15[b] (total 12H, each s), 2.88[b], 3.07[a] (total 3H, both s), 3.37—3.64 (total 2H, 3-H, 4-H), 3.41[b], 3.46[a], 3.50[a], 3.53[b] (total 6H, each s), 4.14—4.62 (total 5H, 1-H, 2-H, 6-H), 5.07[a], 5.22[b] (total 1H, 5-H) (peak area a : b = 5 : 2).

Synthesis of 23 A solution of *N*-acetyl-D-galactosamine (280 mg) in 9% HCl–MeOH (5 ml) was heated under reflux for 3 h and neutralized with 5% KOH–MeOH. The filtrate was evaporated under reduced pressure to give the residue, which was purified by SiO_2 column chromatography [$CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)] to give methyl *N*-acetyl-D-galactosamide (136 mg). A solution of mtehyl *N*-acetyl-D-galactosamide (136 mg) in DMSO (2 ml) was treated with a dimsyl carbanion solution (6 ml) and the whole solution was stirred at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (6 ml) under ice-cooling and then stirred at room temperature for a further 1 h in the dark. Work up of the reaction mixture as described for methylation of sarasinoside A_1 (**1**) gave a product, which was purified by SiO_2 column chromatography (benzene : acetone = 5 : 2) to furnish the fully methylated derivative (102 mg). The fully methylated derivative was treated with 0.5 N H_2SO_4 in 95% acetic acid (2.5 ml) and the whole was heated at 100 °C for 3 h. The reaction mixture was then treated with H_2O (2.5 ml),

heated at 100 °C for an additional 3 h, neutralized with Dowex 1 × 2 OH^- form and filtered. The filtrate was evaporated to dryness under reduced pressure. A solution of the residue in MeOH–H_2O (1 : 1) (3 ml) was treated with $NaBH_4$ (250 mg) and the mixture was stirred at room temperature for 2 h, then treated with glacial acetic acid (0.6 ml). The whole was evaporated under reduced pressure. The residue was treated with acetic anhydride (5 ml) and heated at 100 °C for 2 h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned into $CHCl_3$–H_2O. The $CHCl_3$-soluble portion was evaporated under reduced pressure to give the residue, which was purified by column chromatography (SiO_2, AcOEt) to give 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamido-D-galactitol (**23**) (25 mg).

Synthesis of 24 A solution of *N*-acetyl-D-glucosamine (295 mg) in 9% HCl–MeOH (6 ml) was heated under reflux for 6 h and neutralized with 5% KOH–MeOH. The filtrate was evaporated under reduced pressure to give the residue, which was purified by SiO_2 column chromatography [$CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)] to give methyl *N*-acetyl-D-glucosamide (165 mg). A solution of methyl *N*-acetyl-D-glucosamide in pyridine (5 ml) was treated with trityl chloride (215 mg) and the whole was stirred at 60 °C for 6 h. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ phase was taken and washed with brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (450 mg), which was purified by column chromatography (SiO_2, $CHCl_3$: MeOH = 15 : 1) to give methyl 6-*O*-trityl-*N*-acetyl-D-glucosamide (274 mg). The methyl 6-*O*-trityl-*N*-acetyl-D-glucosamide (274 mg) was treated as described above for methyl *N*-acetyl-D-galactosamide to give 3,4,-di-*O*-methyl-1,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamido-D-glucitol (**24**) (16 mg).

Complete Methylation of Sarasinoside B_1 (5) Followed by Acetolysis, Hydrolysis and Derivation into Partially Methylated Hexitol Acetates 1) A solution of **5** (60 mg) in DMSO (2 ml) was treated with a dimsyl carbanion solution (4 ml) and the whole mixture was stirred at room temperature for 1 h under an N_2 atmosphere, then treated with CH_3I (4 ml) and stirred at room temperature for an additional 1 h in the dark. Work-up of the reaction mixture as described above for methylation of sarasinoside A_1 (**1**) gave the fully methylated derivative (49 mg).

2) The fully methylated derivative (49 mg) was treated with 0.5 N H_2SO_4 in 95% acetic acid (1 ml) and the whole mixture was heated at 80 °C for 5 h. The reaction mixture was then treated with water (1 ml) and heated at 80 °C for an additional 5 h.

3) The reaction mixture was neutralized with Dowex 1 × 2 OH^- form and the filtrate was evaporated to dryness under reduced pressure. A solution of the residue in MeOH–H_2O (1 : 1) (2 ml) was treated with $NaBH_4$ (40 mg) and then stirred at room temperature for 5 h. The reaction mixture was treated with glacial acetic acid (0.5 ml) and the whole was evaporated under reduced pressure. The residue was treated with acetic anhydride (3 ml) and heated at 100 °C for 3 h. Work-up of the reaction mixture as described above for sarasinoside A_1 (**1**) gave 3-*O*-methyl-1,2,4,5-tetra-*O*-acetylxylitol (**19**, 3 mg), 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylglucitol (**20**, 7 mg), 3,4-di-*O*-methyl-1,2,5-tri-*O*-acetylxylitol (**22**, 2 mg), 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidogalactitol (**23**, 4 mg), and 3,4-di-*O*-methyl-1,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol (**24**, 4 mg). 3,4-Di-*O*-methyl-1,2,5-tri-*O*-acetylxylitol (**22**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.07, 2.09, 2.12, 3.48, 3.54 (each 3H, s), 3.49 (1H, m, 3-H), 3.55 (1H, m, 4-H), 4.17 (1H, dd, J = 5.5, 11.8 Hz, 5-H), 4.19 (1H, dd, J = 7.0, 12.2 Hz, 1-H), 4.31 (1H, dd, J = 4.7, 11.8 Hz 5-H), 4.42 (1H, dd, J = 3.7, 12.2 Hz, 1-H), 5.33 (1H, ddd, J = 3.7, 4.9, 7.0 Hz, 2-H).

Acidic Hydrolysis of Sarasinosides A_2 (2), B_2 (7), and C_2 (6) A mixture of **2** (43 mg) and 3% aqueous H_2SO_4 (1 ml) was heated for 2 h under reflux on a water-bath. After dilution with H_2O, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (22 mg), which was purified by column chromatography (SiO_2 treated with $AgNO_3$, *n*-hexane : AcOEt = 5 : 2) to furnish a sapogenol **12**. **12**: mp 136—139 °C (MeOH–AcOEt), colorless needles. $[\alpha]_D$ +8.7° (c = 0.2, $CHCl_3$, 23 °C). UV (MeOH) λ_{max}: Table I. IR (CCl_4) ν_{max} cm^{-1}: 3620, 2923, 2851, 1688, 1445, 1026. CD ($c = 5.7 \times 10^{-2}$, MeOH). Table I. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.55, 0.90 (both 3H, s), 0.95 (3H, d, J = 6.4 Hz, 20-CH_3), 0.98, 1.01 (both 3H, s), 1.89, 2.15 (both 3H, s, 25-CH_3), 3.25 (1H, dd, J = 4.6, 11.6 Hz, 3α-H), 5.40 (1H, m), 5.45 (1H, m), 6.06 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.6 (s, C-23), 153.9 (s, C-25), 147.3 (s, C-9), 136.1 (s, C-8), 124.9 (d, C-24), 121.9 (d, C-7), 117.4 (d, C-11), 78.1 (d, C-3). Mass m/z (%): 424 (54, M^+), 326 (100), 297 (34). High-resolution Mass Calcd for $C_{29}H_{44}O_3$: 424.334. Found: 424.336. Sarasinosides B_2 (**7**) (20 mg) and C_2 (**6**) (18 mg) were hydrolyzed as described above to give the same sapogenol **12**, respectively. Compound **12** thus obtained was shown to be identical with an authentic sample obtained from **2** by TLC and mass spectral comparisons.

Acidic Hydrolysis of Sarasinosides A_3 (3), B_3 (9), C_3 (8) A mixture of **3** (32 mg) and 5% aqueous H_2SO_4–MeOH (1 : 1) (2 ml) was heated for 1 h under reflux. After dilution with H_2O, the reaction mixture was extracted with AcOEt. The AcOEt soluble portion was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract yielded a product (17 mg), which was purified by SiO_2 column chromatography (benzene : acetone = 45 : 1) to give a sapogenol **13**. **13**: mp 135—138 °C (MeOH–AcOEt), colorless needles. $[\alpha]_D$ −10° (c = 0.2, $CHCl_3$, 23 °C). UV (MeOH) λ_{max}: Table I. IR (CCl_4) ν_{max} cm^{-1}: 3628, 2954, 2933, 2853, 1687, 1618, 1032. CD ($c = 6.5 \times 10^{-2}$, MeOH): Table I. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.84, 0.86 (both 3H, s), 0.97 (3H, d, J = 6.4 Hz, 20-CH_3), 1.02, 1.04 (both 3H, s), 1.89, 2.15 (both 3H, s, 25-CH_3), 3.25 (1H, dd, J = 4.6, 11.6 Hz, 3α-H), 5.34 (1H, m, 15-H), 6.08 (1H, s, 24-H). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 200.6 (s, C-23), 153.9 (s, C-25), 151.6 (s, C-14), 142.5 (s, C-9), 125.0 (d, C-24), 123.1 (s, C-8), 117.1 (d, C-15), 77.9 (d, C-3). Mass m/z (%): 424 (75, M^+), 406 (15, $M^+ - H_2O$), 326 (63), 83 (100). High-resolution Mass Calcd for $C_{29}H_{44}O_2$: 424.334. Found: 424.334. Sarasinosides B_3 (**9**) (20 mg) and C_3 (**8**) (15 mg) were hydrolyzed as described above to give the same sapogenol **13**. Compound **13** thus obtained was shown to be identical with an authentic sample obtained from **3** by TLC and mass spectral comparisons.

Catalytic Hydrogenation of Sarasinosides A_1 (1), A_2 (2), and A_3 (3) A suspension of 10% Pd–C (70 mg) in MeOH (4 ml) was stirred under a hydrogen atmosphere for 15 min and **1** (58 mg) was then added to this suspension. The whole mixture was stirred for a further 24 h, then filtered. Removal of the solvent from the filtrate under reduced pressure gave a product (56 mg). HPLC purification (Zorbax ODS, MeOH : H_2O = 5 : 1) of the product furnished **25** (45 mg). **25**: mp 212—215 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −11° (c = 0.3, MeOH, 25 °C). UV (MeOH) λ_{max} nm: transparent above 210 nm. IR (KBr) ν_{max} cm^{-1}: 3360 (br), 2925, 2869, 1640, 1547, 1054 (br). CD ($c = 7.5 \times 10^{-2}$, MeOH): $[\theta]_{221}$ −6600 (neg. max.). ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.61 (3H, s), 0.87 [6H, d, J = 6.7 Hz, 25-$(CH_3)_2$], 0.99 (3H, d, J = 5.8 Hz, 20-CH_3), 1.03, 1.19, 1.31 (each 3H, s), 2.04, 2.09 (both 3H, s, $NHCOCH_3$), 3.15 (1H, dd, J = 4.7, 11.4 Hz, 3α-H), 4.57 (1H, d, J = 7.9 Hz), 5.20 (1H, d, J = 8.2 Hz), 5.21 (1H, d, J = 7.6 Hz), 5.59 (1H, d, J = 7.9 Hz), 5.61 (1H, d, J = 7.3 Hz). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 210.1 (s, C-23), 171.9, 171.5 (both s, $NHCOCH_3$), 136.4 (s, C-9), 127.6 (s, C-8), 106.8 (d), 105.6 (d), 102.7 (d), 102.3 (d), 101.9 (d), 90.2 (d, C-3). *Anal.* Calcd for $C_{62}H_{102}N_2O_{26} \cdot 2H_2O$: C, 56.09; H, 8.05; N, 2.11. Found: C, 56.12; H, 7.85; N, 2.19. Sarasinosides A_2 (**2**) (17 mg) and A_3 (**3**) (21 mg) were hydrogenated over 10% Pd–C under similar conditions to give **25** (13 mg from **2**, 16 mg from **3**). Compound **25** thus obtained was shown to be identical with an authentic sample obtained from **1** by mixed melting-point determination, HPLC, and ^{13}C-NMR comparisons.

Catalytic Hydrogenation of Sarasinosides B_1 (5), B_2 (7), and B_3 (9) A suspension of 10% Pd–C (17 mg) in MeOH (4 ml) was stirred under a hydrogen atmosphere for 15 min. Compound **5** (12 mg) was added to this suspension and the reaction mixture was stirred for a further 24 h, then filtered. Removal of the solvent from the filtrate under reduced pressure gave a product (11 mg). HPLC purification (Zorbax ODS, MeOH : H_2O = 5 : 1) of the product furnished **27** (8 mg). **27**: mp 200—203 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −19° (c = 0.2, MeOH, 25 °C). UV (MeOH) λ_{max} nm: transparent above 210 nm. IR (KBr) ν_{max} cm^{-1}: 3350 (br), 2923, 2860, 1646, 1548, 1039 (br). CD ($c = 8.1 \times 10^{-2}$, MeOH): $[\theta]_{220}$ −6900 (neg. max). ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.61 (3H, s), 0.86, 0.87 [both 3H, d, J = 6.7 Hz, 25-$(CH_3)_2$], 0.98 (6H, br s), 1.16, 1.32 (both 3H, s), 2.05, 2.10 (both 3H, s, $NHCOCH_3$), 3.15 (1H, dd, J = 4.6, 11.0 Hz), 4.56 (1H, d, J = 7.9 Hz), 5.20 (1H, d, J = 8.2 Hz), 5.21 (1H, d, J = 7.3 Hz), 5.38 (1H, d, J = 7.3 Hz), 5.56 (1H, d, J = 8.6 Hz). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 210.1 (s, C-23), 172.3, 171.9 (both s, $NHCOCH_3$), 136.3 (s, C-9), 127.6 (s, C-8), 106.6 (d), 105.3 (d), 103.2 (d), 102.6 (d), 101.8 (d), 90.0 (d, C-3). *Anal.* Calcd for $C_{61}H_{100}N_2O_{25} \cdot H_2O$: C, 57.26; H, 8.03; N, 2.19. Found: C, 57.42; H, 7.99, N, 2.35. Sarasinosides B_2 (**7**) (14 mg) and B_3 (**9**) (20 mg) were hydrogenated over 10% Pd–C under similar conditions to give **27** (10 mg from **7**, 16 mg from **9**). Compound **27** thus obtained was shown to be identical with an authentic sample obtained from **5** by mixed melting-point determination, HPLC, and ^{13}C-NMR comparisons.

Catalytic Hydrogenation of Sarasinosides C_1 (4), C_2 (6), and C_3 (8) A

suspension of 10% Pd–C (100 mg) in MeOH (7 ml) was stirred under a hydrogen atmosphere for 15 min. Compound **4** (150 mg) was added to this suspension and the reaction mixture was stirred for a further 24 h, then filtered. Removal of the solvent from the filtrate under reduced pressure gave a product (120 mg). HPLC purification (Zorbax ODS, MeOH: $H_2O = 5:1$) of the product furnished **26** (100 mg): mp 197—200 °C (MeOH–H_2O), fine crystals. $[\alpha]_D$ −20° ($c = 1.2$, MeOH, 25 °C). UV (MeOH) λ_{max} nm: transparent above 210 nm. IR (KBr) ν_{max} cm^{-1}: 3360 (br), 2927, 2865, 1638, 1548, 1037 (br). CD ($c = 6.0 \times 10^{-2}$, MeOH): $[\theta]_{222}$ −6200 (neg. max.). ^{1}H-NMR (500 MHz, d_5-pyridine + D_2O) δ: 0.61 (3H, s), 0.86 (3H, d, $J = 6.7$ Hz, 25-CH_3), 0.87 (3H, d, $J = 6.4$ Hz, 25-CH_3), 0.95 (3H, s), 0.99 (3H, d, $J = 5.5$ Hz, 20-CH_3), 1.13, 1.28 (both 3H, s), 2.09, 2.10 (both 3H, s, $NHCOCH_3$), 3.18 (1H, dd, $J = 4.1$, 11.8 Hz, 3α-H), 4.64 (1H, d, $J = 7.0$ Hz), 4.96 (1H, d, $J = 7.3$ Hz), 5.12 (1H, d, $J = 8.6$ Hz), 5.44 (1H, d, $J = 8.5$ Hz). ^{13}C-NMR (125 MHz, d_5-pyridine) δ_C: 210.2 (s, C-23), 172.3, 171.6 (both s, $NHCOCH_3$), 136.4 (s, C-9), 127.7 (s, C-8), 105.3 (d), 104.6 (d), 102.3 × 2 (d), 89.4 (d, C-3). *Anal.* Calcd for $C_{55}H_{90}N_2O_{20} \cdot H_2O$: C, 59.23; H, 8.13; N, 2.51. Found: C, 59.11; H, 8.20; N, 2.38. Sarasinoside C_2 (**6**) (50 mg) and C_3 (**8**) (20 mg) were hydrogenated over 10% Pd–C under similar conditions to give **26** (30 mg from **6**, 14 mg from **8**). Compound **26** thus obtained was shown to be identical with an authentic sample obtained from **4** by mixed melting-point determination, HPLC, and ^{13}C-NMR comparisons.

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